Topical Review

Functions of AMP-activated protein kinase in adipose tissue

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AMP-activated protein kinase (AMPK) is involved in cellular energy homeostasis. Its functions have been extensively studied in muscles and liver. AMPK stimulates pathways which increase energy production (glucose transport, fatty acid oxidation) and switches off pathways which consume energy (lipogenesis, protein synthesis, gluconeogenesis). This has led to the concept that AMPK has an interesting pharmaceutical potential in situations of insulin resistance and it is indeed the target of existing drugs and hormones which improve insulin sensitivity. Adipose tissue is a key player in energy metabolism through the release of substrates and hormones involved in metabolism and insulin sensitivity. Activation of AMPK in adipose tissue can be achieved through situations such as fasting and exercise. Leptin and adiponectin as well as hypoglycaemic drugs are activators of adipose tissue AMPK. This activation probably involves changes in the AMP/ATP ratio and the upstream kinase LKB1. When activated, AMPK limits fatty acid efflux from adipocytes and favours local fatty acid oxidation. Since fatty acids have a key role in insulin resistance, especially in muscles, activating AMPK in adipose tissue might be found to be beneficial in insulin-resistant states, particularly as AMPK activation also reduces cytokine secretion in adipocytes.

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Adipose tissue: energy and endocrinology

Storage of energy when food is available is a determinant of survival in periods of increased energy expenditure or decreased energy availability. Quantitatively, the main form of energy storage is represented by triglycerides in the adipocytes of adipose tissue. Adipose tissue is also composed of endothelial cells, fibroblasts and macrophages which are found in the stroma–vascular fraction. New adipocytes can be formed throughout life from this fraction depending upon the nutritional and hormonal conditions. The origin of the lipids stored can be either the diet or de novo synthesis from non-lipid substrates (lipogenic process). This process is active in rodent adipose tissue but relatively minor in human adipose tissue. In order to take up lipids from plasma, adipocytes synthesize a specific enzyme called lipoprotein lipase which is exported to the luminal side of vascular endothelium where it can hydrolyse triglyceride-rich lipoproteins such as chylomicrons and VLDLs (very low density lipoproteins) to yield fatty acids and free glycerol. Fatty acids enter into the adipocytes through transporters and are re-esterified with glycerol phosphate to form triglycerides stored in a single lipid droplet in white adipocytes. This droplet is surrounded by a membrane, itself covered with a protein called perilipin found exclusively in adipocytes. When needed, triglycerides are hydrolysed (lipolysis) into fatty acids and glycerol which are exported back into the blood. Lipolysis requires several enzymes acting successively and key enzymes are adipose tissue triglyceride lipase and hormone-sensitive lipase (HSL). HSL activity is regulated acutely through several mechanisms including reversible phosphorylation and translocation from the cytosol to the surface of the lipid droplet. Insulin favours lipid storage through the activation of lipogenesis, lipoprotein lipase synthesis and export to the vascular endothelium, and triglyceride esterification through the production of glycero-phosphate from glucose. In contrast adrenergic hormones produced either by the adrenal medulla or by the local sympathetic innervation activate lipolysis through binding to a β-agonist receptor and production of cAMP.

It is now well appreciated that, in addition to its functions related to energy storage and release, adipose

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tissue is also an endocrine organ, strongly involved in overall energy homeostasis and substrate partitioning. The most important hormones produced by adipose tissue are leptin and adiponectin. Leptin is a cytokine produced in proportion to the amount of adipose tissue and which acts in specific brain hypothalamic nuclei to reduce food intake and in rodents to activate thermogenesis (Friedman, 2000). Leptin also has actions outside of the brain, one of which is to stimulate fatty acid oxidation in muscles and liver, at least in part through AMP-activated protein kinase (AMPK) activation (Minokoshi *et al.* 2002).

Adiponectin belongs to the complement 1q family. It is one of the most abundant transcripts in adipocytes and its plasma concentration is high. It circulates and signals as a homomultimer. In contrast to leptin, its secretion and plasma concentration are inversely related to adiposity. Plasma adiponectin concentrations are decreased in obese and type 2 diabetic rodents, primates and humans (Tsao *et al.* 2002). Adiponectin is considered to be an insulin-sensitizing hormone since it activates muscle glucose utilization but also induces muscle and hepatic fatty acid oxidation (accumulation of fatty acids or fatty acyl-CoAs in insulin-sensitive cells is deleterious for insulin signalling) and decreases hepatic glucose production (Fruebis *et al.* 2001; Berg *et al.* 2002; Matsuzawa *et al.* 2004). It has been shown, at least in the liver, that adiponectin effects require AMPK activation (Yamauchi *et al.* 2002). Cytokines such as interleukin-6 (IL-6) and tumour necrosis factor (TNFα) are produced by adipose tissue although probably not specifically by adipocytes but also by cells from the stroma–vascular fraction and can favour insulin resistance in insulin-sensitive tissues.

AMPK is involved in other tissues in the maintenance of cellular as well as body energy homeostasis. When activated, it inhibits energy-consuming processes and activates energy-producing processes (see other contributions in this issue). Adipose tissue is a major component of energy homeostasis and a key player in the regulation of insulin sensitivity through fatty acid release and hormone secretion. Understanding the function of AMPK in adipocytes is thus crucial for assessing the importance of this enzyme in overall energy metabolism.

Structure of AMPK in adipose tissue

AMPK exists in the cell as a heterotrimeric complex with a catalytic (α) and two regulatory subunits (β and γ) (Woods *et al.* 1996*a*). Several isoforms have been identified for each subunit ($\alpha1$, $\alpha2$, $\beta1$, $\beta2$, $\gamma1$, $\gamma2$, $\gamma3$), that can lead to the formation of 12 different complexes. These combinations confer different properties to the AMPK complexes (Hardie & Carling, 1997) and show relative tissue specificity (Cheung *et al.* 2000). Muscle cells mainly express AMPK complexes containing the $\alpha2$ catalytic subunit and liver expresses both $\alpha1$ and $\alpha2$ isoforms (Stapleton *et al.* 1996; Woods *et al.* 1996*b*). In adipose tissue, the $\alpha1$ catalytic subunit is the predominant isoform expressed and accounts for the major part of AMPK activity (Lihn *et al.* 2004; Daval *et al.* 2005). Although the functional significance of these different complexes remains unclear, it can be emphasized that AMPK complexes containing the $\alpha1$ isoform are less sensitive to AMP (Salt *et al.* 1998). At present there is no data concerning the respective expression of other AMPK subunits in adipocytes.

Regulation of AMPK in adipose tissue

In adipose tissue, fasting and exercise activate AMPK (Park *et al.* 2002; Daval *et al.* 2005; Sponarova *et al.* 2005). Since both situations are concomitant with adrenergic stimulation, it could be anticipated that β-adrenergic agonists and their second messenger cAMP would stimulate AMPK activity. This is indeed the case (Haystead *et al.* 1990; Moule & Denton, 1998; Daval *et al.* 2005; Sponarova *et al.* 2005). It has been suggested that the effect of exercise on adipose tissue AMPK could also be secondary to the secretion of IL-6 by muscles (Kelly *et al.* 2004). Indeed, IL-6 is able to activate AMPK in F442A adipocytes and a decreased AMPK phosphorylation is found after exercise in adipose tissue of IL-6 knock-out mice.

Leptin (Orci *et al.* 2004) and adiponectin (Wu *et al.* 2003; Sell *et al.* 2006) are able to activate AMPK in adipose tissue. Hypoglycaemic drugs such as biguanides are also inducing an increase of AMPK activity in adipocytes (Daval *et al.* 2005; Huypens *et al.* 2005). More controversial results are found using thiazolidinediones, another class of hypoglycaemic agents which are ligands of the transcription factor PPARγ since Huypens *et al.* were unable to detect AMPK activation in 3T3-L1 adipocytes using 10 μM troglitazone (Huypens *et al.* 2005) whereas an increased AMPK activity was shown *in vivo* in adipose tissue of rats treated with pioglitazone (Saha *et al.* 2004) or rosiglitazone (Ye *et al.* 2004). In our own experiments, AMPK was activated by thiazolidinediones in isolated adipocytes but at concentrations higher than 200 μM (authors' unpublished results).

AMP and ATP concentrations in the cell are closely related due to the presence of the enzyme adenylate kinase. An increase in AMP is an exquisitely sensitive indicator of a decrease in the level of cellular energy charge. AMP activates AMPK by a complex mechanism involving allosteric effects and more importantly, the phosphorylation by upstream protein kinases of the threonine residue 172 within the activation loop of the α catalytic subunit (Hardie & Carling, 1997). Two upstream kinases have been characterized. LKB1 is a kinase which

is constitutively active and phosphorylates AMPK when AMP concentration rises in the cell and binds to the γ subunit, thus transforming AMPK in a suitable substrate for LKB1 (Hawley *et al.* 2003; Woods *et al.* 2003; Shaw *et al.* 2004). The second kinase, calmodulin kinase kinase β, phosphorylates and activates AMPK in the presence of an increased calcium concentration, independently of an increase in AMP concentration (Hawley *et al.* 2005; Woods *et al.* 2005). In adipose tissue, several indirect arguments suggest that LKB1 is involved in AMPK activation. Treatment of adipocytes with AICAR, a drug which is transformed in the cell into ZMP, an analogue of AMP, activates AMPK in adipocytes (Sullivan *et al.* 1994; Corton *et al.* 1995; Salt *et al.* 2000; Lihn *et al.* 2004; Daval *et al.* 2005). In addition phenformin, a biguanide, induces AMPK activation and decreases ATP concentration (Daval *et al.* 2005). In transgenic mice expressing an uncoupling protein (UCP1) in white adipose tissue, the AMP/ATP ratio is increased and AMPK is activated (Matejkova *et al.* 2004). Finally, β-adrenergic lipolytic agents which induce AMPK stimulation are concomitant with a decrease in ATP concentration (Angel *et al.* 1971; Issad *et al.* 1995). To the best of our knowledge, a potential role of calmodulin kinase kinase β in AMPK activation has not been demonstrated in adipocytes.

In conclusion, several situations which activate AMPK in adipose tissue are concomitant with an increased AMP/ATP ratio, strongly suggesting the involvement of the upstream kinase LKB1 (Fig. 1).

AMPK and adipocyte differentiation

Indirect evidence suggests that AMPK activation can inhibit preadipocyte differentiation. AICAR treatment of 3T3-L1 or F442A preadipocytes inhibits adipocyte differentiation and blocks the expression of late adipogenic markers such as fatty acid synthase and the transcription factors PPARγ and C/EBPα and promotes apoptosis (Habinowski & Witters, 2001; Dagon *et al.* 2006). This is not totally unexpected if one considers that differentiation is an energy-consuming process involving new membrane (and thus lipid) and protein synthesis, two pathways which are strongly inhibited by AMPK activation (Bolster *et al.* 2002; Horman *et al.* 2002). However, in mice lacking the catalytic α1 subunit, the main catalytic isoform present in adipose tissue, the differentiation potential does not seem to be grossly affected since adipocytes are smaller but not more numerous (Daval *et al.* 2005). It is thus not clear whether AMPK has a physiological regulatory function in adipocyte differentiation.

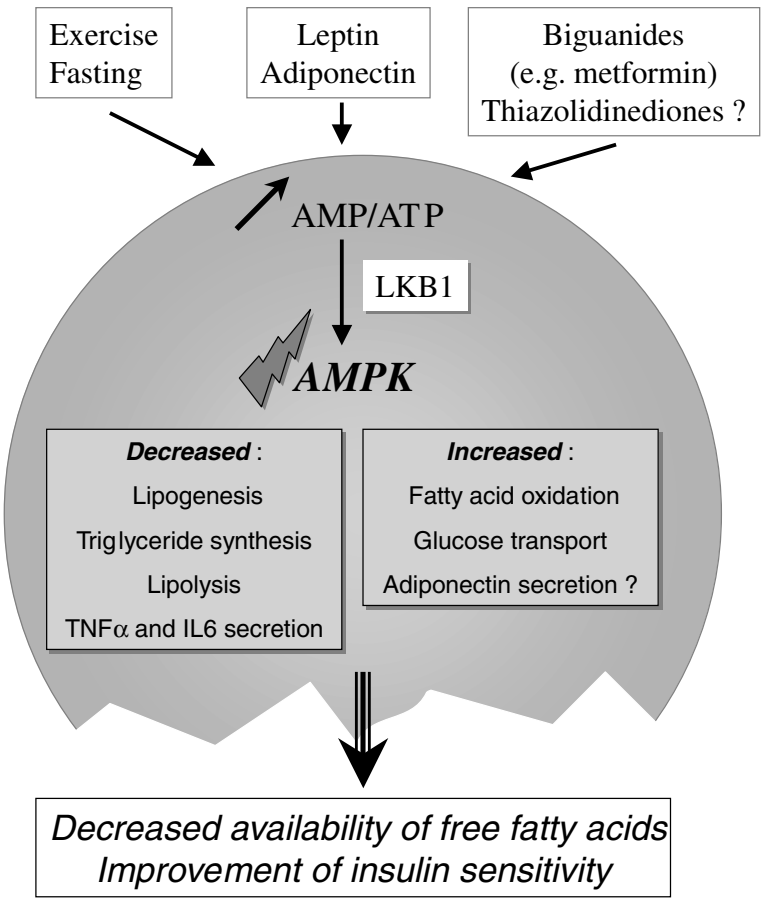

Figure 1. AMPK activation in adipose tissue and its intracellular actions

AMPK and regulation of adipose tissue metabolism

Once activated, AMPK phosphorylates a number of proteins and modulates the transcription of genes implicated in the regulation of energy metabolism to switch on catabolic pathways that produce ATP and switch off anabolic pathways that consume ATP.

Lipogenesis and triglyceride synthesis. One of the first proteins identified as a target of AMPK was acetyl-CoA carboxylase (ACC) which synthetizes malonyl-CoA from acetyl-CoA and is a key enzyme of the lipogenic pathway (Sim & Hardie, 1988). The phosphorylation and thus inhibition of ACC by AMPK has been shown in several studies *in vivo* and in intact cells. In adipocytes, a direct effect of AMPK activation on ACC phosphorylation and activity was shown in rodent adipocytes using either AICAR (Sullivan *et al.* 1994) or expression of a constitutively active AMPK (Daval *et al.* 2005). This was concomitant with a decreased lipogenic rate (Sullivan *et al.* 1994). Conversely, over-expression of a dominant negative form of AMPK in adipocytes precludes the phosphorylation of ACC after AICAR or isoproterenol (isoprenaline) treatment (Daval *et al.* 2005). Exercise, which activates AMPK in adipose tissue is concomitant with a decreased ACC activity and malonyl-CoA concentration (Park *et al.* 2002). Exercise in rats also induces an increase in malonyl-CoA decarboxylase activity, thus further reducing malonyl-CoA concentrations, and a decrease in glycerol-acyl transferase activity, an enzyme involved in triglyceride synthesis. These effects are mimicked by AICAR treatment of the animals. As in the liver (Foretz *et al.* 1998; Leclerc *et al.* 1998), an activation of AMPK in adipocytes is concomitant with a decreased expression of lipogenic enzyme mRNA (Orci *et al.* 2004).

In conclusion, activation of AMPK in rodent adipocytes leads to a decreased lipogenic flux and a decreased triglyceride synthesis.

Lipolysis. The other major function of adipose tissue is the breakdown of triglycerides through the lipolytic pathway that occurs during fasting to provide fatty acids and glycerol as fuels for peripheral tissues. In adipocytes, AMPK activation using AICAR has been shown to inhibit β-adrenergic-induced lipolysis (Sullivan *et al.* 1994; Corton *et al.* 1995). Recent work (Daval *et al.* 2005) has confirmed these studies in a more direct way, showing that overexpression of a constitutively active AMPK in rat adipocytes was indeed inhibiting isoproterenol-induced lipolysis, whereas overexpression of a dominant negative form of AMPK had a converse effect. Other activators of AMPK such as biguanides also had an inhibitory action on lipolysis (Daval *et al.* 2005). These results are at variance with the study of Yin *et al.* in 3T3-L1 adipocytes (Yin *et al.* 2003) since these authors have shown that overexpression of a dominant negative form of AMPK inhibits isoproterenol-induced lipolysis suggesting, rather, a lipolytic action of AMPK activation. However, AMPK activity was not measured in these conditions and thus final conclusions from these experiments are difficult. Using the same cell line, we have demonstrated that AICAR and phenformin induce AMPK activity and strongly impair lipolysis (Daval *et al.* 2005). Interestingly, in mice lacking the predominant α1 AMPK isoform, the size of adipocytes is reduced and basal and isoproterenol-induced lipolysis is higher than that of control adipocytes (Daval *et al.* 2005). This argues in favour of an inhibitory role of AMPK activation on lipolysis.

Mice with a general knock-out of the AMPK α2 catalytic subunit have an increase in adipose mass due to adipocyte hypertrophy when fed a high fat diet and compared with high fat fed control mice (Villena *et al.* 2004). Since the AMPK α2 subunit represents only a very minor part of AMPK activity in adipose tissue, this adipocyte hypertrophy may be the consequence of the adaptation of adipose metabolism subsequent to the loss of AMPK α2 activity in other tissues such as muscle or liver.

What could be the mechanism accounting for lipolysis inhibition by AMPK? At present, two rate-limiting enzymes controlling the hydrolysis of triglycerides in adipocytes have been described. Hormone-sensitive lipase (HSL) was the first one to which a regulatory role was ascribed. HSL hydrolyses triglycerides, diglycerides and cholesteryl esters, although it has a much higher specific activity for diglycerides. Lipolytic agents such as β-adrenergic agonists acutely regulate HSL by increasing cAMP levels in the cell, thus activating cAMP-dependent protein kinase (protein kinase A; PKA) which in turn phosphorylates HSL and increases its intrinsic activity as well as promoting its translocation to the lipid droplet (Yeaman, 2004). HSL is a substrate for AMPK (Garton & Yeaman, 1990). AMPK phosphorylates Ser-565, precluding the further phosphorylation of the regulatory Ser-563 by PKA. Although it was later suggested that the true regulatory serines phosphorylated by PKA of HSL were Ser-659 and Ser-660 (Anthonsen *et al.* 1998), we have confirmed that activation of AMPK increases HSL phosphorylation on Ser-565 in adipocytes and more importantly that it precludes its isoproterenol-induced translocation to the lipid droplet, a major requirement for lipolysis activation (Daval *et al.* 2005).

The existence of a second regulatory lipase was discovered during the studies of HSL knock-out mice (Osuga *et al.* 2000; Wang *et al.* 2001; Haemmerle *et al.* 2002). In these mice, the amount of adipose tissue is

decreased and its lipid composition is affected with a marked diacylglycerol accumulation. β-Agonist-induced lipolysis is also lower. However, basal lipolysis is normal and although a cholesteryl ester hydrolase activity is no longer detectable in the adipose tissue of HSL knock-out mice, a neutral triglyceride lipase activity is still present. This activity was identified as an adipose triglyceride lipase (ATGL) (Zimmermann *et al.* 2004). Recently, the genetic deletion of this ATGL has confirmed its importance in triglyceride hydrolysis as well as the likely role of HSL as a diglyceride (rather than a triglyceride) hydrolase (Haemmerle *et al.* 2006). Interestingly, in HSL knock-out mice, the residual triglyceride lipase activity (ATGL) is increased in the presence of β-agonists and part of this lipolytic response could be secondary to a translocation from the cytoplasm to the lipid droplet, as shown previously for HSL (Okazaki *et al.* 2002). Thus, it is obviously of interest to test whether ATGL is also a substrate for AMPK and whether its phosphorylation precludes its translocation to the lipid droplet.

The lipid droplet membrane is covered with perilipin, a hydrophobic phosphoprotein. Phosphorylation of perilipin by PKA induces its relocation away from the lipid droplet membrane, allowing HSL (and probably ATGL) to reach its substrates (Tansey *et al.* 2004). Perilipin knock-out mice have indeed an enhanced basal lipolysis. Whether perilipin is a target of AMPK which when phosphorylated by this enzyme would be unable to relocate away from the droplet membrane is presently unknown.

To summarize, AMPK is activated in conditions of increased lipolysis such as exercise and fasting. This activation inhibits fatty acid and triglyceride synthesis and could limit lipolysis. This latter finding might seem counter-intuitive if one considers AMPK as an enzyme which in case of energy shortage should rather enhance energy availability (here fatty acids through lipolysis) for cells. However, a high rate of lipolysis could be very demanding for adipocyte energy homeostasis since part of the fatty acids can be reactivated into acyl-CoA, a reaction which consumes ATP and generates AMP. Alternatively, accumulation of free fatty acids into the adipocyte could be deleterious for energy-producing processes since they are well-known mitochondrial uncouplers (Kadenbach, 2003). Activation of AMPK would then be a feedback mechanism limiting the cellular energy drain associated with lipolysis in adipocytes.

Fatty acid oxidation. Two models of AMPK activation in adipose tissue are concomitant with an increased fatty acid oxidation. In the first one, the uncoupling mitochondrial protein UCP-1 is overexpressed in adipocytes leading to an increase in the AMP/ATP ratio, activation of AMPK, inactivation of ACC and a decreased lipogenesis (Matejkova *et al.* 2004). This induces an increased capacity for fatty acid oxidation which could be due to a decreased concentration of malonyl-CoA, alleviating the inhibition on carnitine palmitoyl-transferase I which catalyses the entry of fatty acids in mitochondria and constitutes the rate-limiting enzyme of fatty acid oxidation. UCP-1 overexpression is also concomitant with mitochondrial biogenesis in adipocytes (Rossmeisl *et al.* 2002). Interestingly, these mice are resistant to nutrient-induced obesity.

In a second model, Orci and coworkers (Orci *et al.* 2004) have induced hyperleptinaemia using an adenoviral-mediated overexpression of leptin in the liver. In adipose tissue of these hyper-leptinized rats, UCP-1 and UCP-2 expression is increased, AMPK activity is induced and leads to the phosphorylation and inactivation of ACC. There is also a strong mitochondrial biogenesis, features that could lead to the 'rapid transformation of white adipocytes into fat-oxidizing machines' (Orci *et al.* 2004). In these animals, hyperleptinaemia induces a depletion in body fat stores (Shimabukuro *et al.* 1997) and the authors suggest that this is due to oxidation of fatty acids within the adipocytes inasmuch as these adipocytes release glycerol but no fatty acids (Wang *et al.* 1999). Interestingly, leptin had no effect during diet-induced obesity implying a leptinergic blockade in adipocytes during overnutrition.

Although activation of AMPK is probably not responsible for all the metabolic characteristics of these models, the results nevertheless suggest that, similar to its effects in other tissues, AMPK activation in adipocytes induces increased fatty acid oxidation.

Glucose transport. AMPK activation stimulates glucose transport through increased GLUT4 translocation in muscles (Kahn *et al.* 2004). Only a few studies have addressed the potential role of AMPK in glucose uptake in adipose cells. Studies performed in 3T3-L1 adipocytes have reported that treatment of differentiated adipocytes with AICAR enhances basal glucose uptake by a mechanism independent of insulin signalling (Salt *et al.* 2000; Sakoda *et al.* 2002). However, overexpression of a dominant negative form of AMPK in 3T3-L1 adipocytes treated with AICAR abolishes AMPK activation without affecting the increase in glucose uptake (Sakoda *et al.* 2002), raising the question of a direct involvement of AMPK in AICAR-stimulated glucose transport in this model. A third study performed in primary rat adipocytes has shown that adiponectin activates AMPK and increases glucose uptake (Wu *et al.* 2003). In this study, the inhibition of AMPK by pharmacological compounds abolishes the adiponectin-stimulated glucose transport and it occurs without affecting insulin-stimulated glucose uptake. This suggests a role of AMPK in glucose transport in adipocytes which could involve a mechanism independent of the insulin signalling pathway. However, whether AMPK

induces the translocation of GLUT4 to the membranes of adipocytes remains unclear.

AMPK and adipokine secretion

As stated above, adipose tissue is now considered as an endocrine organ involved in energy homeostasis, food intake and inflammation. In human adipose tissue, AICAR has been shown to increase the expression of the insulin-sensitizing hormone adiponectin (Lihn *et al.* 2004; Sell *et al.* 2006). A study performed in 3T3-L1 has shown the converse, reporting an inhibition of adiponectin expression and secretion in response to AMPK activation by the anti-diabetic drug metformin (Huypens *et al.* 2005). Metabolic and insulin-sensitizing effects of metformin have been shown to be in part mediated through the activation of AMPK (Zhou *et al.* 2001). However, type 2 diabetic patients treated with metformin display no change in serum adiponectin concentration or adipocyte adiponectin content (Phillips *et al.* 2003; Tiikkainen *et al.* 2004). The role of AMPK in the regulation of adiponectin expression and secretion thus remains unclear.

In human adipose tissue, inhibitory effects of AICAR on the expression and secretion of two pro-inflammatory cytokines, TNFα and interleukin-6 (IL-6) have been reported (Lihn *et al.* 2004; Sell *et al.* 2006). Since TNFα inhibits adiponectin expression (Kappes & Loffler, 2000), it has been suggested that the decrease in TNFα protein may be involved in an up-regulation of adiponectin expression and that the effects of AICAR on adiponectin may be indirect. The inhibition of TNFα and IL-6 secretion by AMPK could be beneficial, since inflammation is thought to contribute to the development of disorders associated with obesity such as insulin resistance. Activation of AMPK in adipose tissue by decreasing TNFα and IL-6 and indirectly increasing adiponectin secretion may thus contribute to the prevention or counteraction of insulin resistance in obese patients. However, the demonstration of a more direct effect of AMPK on cytokine secretion awaits additional experiments.

Conclusion

In the liver, AMPK is part of a mechanism which coordinates changes of lipid metabolism from anabolism to catabolism in case of energy shortage. It includes an inhibition of lipid synthesis and an increased lipid oxidation mediated by a decreased malonyl-CoA content due to an inhibition of ACC activity (Assifi *et al.* 2005). In adipocytes, a similar role for AMPK is conceivable in case of energy shortage or higher energy demand (exercise) since in these situations the observed activation of AMPK can lead to an inhibition of fatty acid synthesis and an activation of fatty acid oxidation. However, in adipocytes AMPK also inhibits lipolysis (Fig. 1). All these actions of AMPK will tend to decrease the availability of fatty acids in the plasma. Since fatty acids have a key role in the onset of insulin resistance, especially in muscles, activating AMPK in adipose tissue might be extremely beneficial in insulin-resistant states such as type 2 diabetes, particularly as AMPK activation also reduces inflammatory cytokine secretion in adipocytes. A number of questions remain nevertheless unsolved: Can AMPK be stimulated in adipose tissue by non-AMP-dependent mechanisms? Is there any difference in AMPK distribution and responsiveness in subcutaneous and deep visceral adipose tissues (an important question if one considers that visceral fat might be a preferred target)? Since many of the studies have been performed on rodent adipocytes or cell lines do they really apply to human adipocytes?

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